

however, to be very similar in mineral character, with no great apparent pauses between the eruptions, which came doubtless from the same centre.

If, as most think, our alluvial diggings are of recent Tertiary times, the superimposed basalt may be more modern than many others. Yet even there has been time for soil for a forest clothing. I have elsewhere alluded to the deep masses of calcareous rock between two beds of Portland basalt. The existence of hundreds of feet of rock, consisting chiefly of the debris of a coralline sea, would assume considerable antiquity for the first basalt.

In New South Wales the basalt is very common in the coal-fields. Count Strzelcecki gives four distinct epochs for the Tasmanian basalts. When standing by the celebrated opalized tree, near the Derwent, I observed that deep beds of basalt lay upon a broad sheet of greenstone. If Victoria differs from Tasmania in its rich deposits of ash, it is inferior to the island beauty in the relative extent and variety of its more consolidated volcanic products.

In conclusion, if we have no *past* glories of greatness, like Rome, to associate with our Victorian volcanic rocks, let us build up with them a bright and happy *future*. A numerous and gladdened peasantry may till our tufa fields. Works of material progress, structures of architectural beauty, haunts of science and the arts, schools of learning, fanes of piety, and homes of free and virtuous citizens, may stand before our children, and our children's children, in the everlasting basalt of our rocks—a type of that stability of being found in truth, in peace, in love.

ART. V.—*Experiments and Observations on Absorption.*

By GEORGE B. HALFORD, M.D., Professor of Anatomy, Physiology, and Pathology, University of Melbourne.

[Read 11th June, 1866.]

I purpose to lay before the Society the results of some experiments upon the absorption of colouring matters by the living body. It is well known that absorption takes place readily when fluids are thrown into the loose connective tissue beneath the skin, or into the serous cavities, as also from the great mucous tracts extending from the eyelids to the lungs, and from the ears to the anus. From the skin also,

especially where thinnest, absorption takes place. By the term absorption, I mean the simple passage of fluids, without retardation for the purposes of assimilation or rejection.

In order to trace the passage of these fluids, I have used the aniline dyes, viz., magenta, mauve, blue, green, cerise, and yellow.

Dr. Roberts, of Manchester, first called the attention of the Royal Society of England, Feb. 18th, 1863, to the wonderful tinting power of magenta, and to the remarkable fact, that if a small quantity be added to a drop of mammalian blood, a minute spot, coloured with the dye, appears at some part of the edge of the disk. Subsequent experiments with tannin resulted in his producing in the red corpuscle of mammals, birds, reptiles, and fishes, "a bright, highly refractive bud or projection on the surface." These he called "pullulations." In the *Quarterly Journal of Microscopical Science* for July 1863, is a *resumé* of his discoveries.

I have long since verified all that Dr. Roberts has stated, and have used magenta dye very extensively in histological inquiry. The effect of the direct application of a solution of magenta to a drop of human blood is, besides producing the pullulation in the corpuscle, to colour the nucleus or contents of the white corpuscle. These white corpuscles are not all of the same size, some containing a single nucleus, others two, three, four, or more. The lymph corpuscle smaller than the white corpuscle, is also deeply tinged, and there is apparently a transitional form of the lymph corpuscle towards the white; and lastly, granular matter deeply tinged.

The blood of the bird or frog has the nucleus of the red corpuscle deeply dyed, whereas the white corpuscles are uncoloured.

Moreover, magenta deeply tinges the following elementary structures :

Corpuscles of lymph and chyle, contents of duodenal epithelium and mucus.

Nucleus of all forms of epithelium.

All young cells.

Envelope and nucleus of some forms of fat.

Nucleus of tendon and of unstriped and striped muscular fibre.

Cell and nucleus of cartilage.

Cell and nucleus of bone, and of red marrow of bone.

All forms of nerve cells.

Nuclei of capillaries and connective tissue.

Having ascertained that applying magenta to the conjunctiva produced no irritation, I hoped that its gradual absorption into the system might reveal to us certain particles and hitherto ill-defined forms of structure, and that possibly the passage of such particles (germinal matter of Beale, histogenetic molecules of Bennett), might be traced from one part of the body to another; but I must say that a great deal of labour has brought me a very little way on, still I am not without hope that I may yet lay something more definite on this subject before the Society.

It matters not whether these dyes are injected into the peritoneal cavity or beneath the skin, they almost immediately make their appearance in the urine. Very little inconvenience attends the operations, and even after injection into the pleural cavity life is not endangered. Thus I have injected the same animal or bird eight and ten times; if care be not used, however, inflammation is produced, most probably from the point of the instrument wounding some of the viscera. In the two cases where death resulted, the peritoneal cavity was found full of *uncoloured* serum and lymph, at the same time that the dye had disappeared from the urine before death.

We have now to determine by what route the dye passes from the abdomen or from beneath the skin to the kidneys; certainly not directly. Knowledge familiar to us all is against such a view. To appear, therefore, in the urine, it must have been brought from the heart by the arteries to the kidneys. Our most direct course is to examine the contents of heart soon after injecting the dyes. For this purpose I performed the following experiments:

Experiment 1.—A frog was injected beneath the skin with equal parts of blue dye and water. Four hours after I exposed the heart, *without disturbing the pericardium*, and saw the blood deeply dyed, flowing into and out of the heart with each relaxation and contraction of the ventricle. The blood in the femoral artery was also dyed, as were all the tissues more or less.

Experiment 2.—Another frog was injected with yellow dye. In twenty minutes, by the same proceeding as above, the circulation of yellowish blood was seen. All the tissues slightly tinged.

Experiment 3.—Magenta dye similarly used, and magenta-coloured blood seen circulating.

Experiment 4.—Green dye produced no effect. I found it unabsorbed beneath the skin.

Having traced these colours from beneath the skin to the heart in the frog, rendered it unnecessary to perform the same experiments with a dog or fowl, in either of which it would be more difficult and perhaps impossible to see coloured blood at the centre of the circulation, through the denser fibres of the heart; whereas, in the frog, the delicate structure of this organ permits of the colour of the blood being easily seen through it. This is a very beautiful experiment, and I recommend it to the notice of physiologists as showing clearly one form of absorption, and, *provided the pericardium be not opened*, the heart's action. We have now to consider by what means the dyes reach the heart.

It has been before stated that the lymph and chyle corpuscles are readily and deeply dyed by magenta, and as by injecting into the peritoneum the dye is brought into close proximity to the lacteals, and hence most liable to absorption by them, I performed numerous experiments upon dogs and fowls, and having killed them as soon as the dyes appeared in the urine, laid bare the thoracic duct, as I had previously the heart in frogs, to see, its coat being transparent, if its contents, the chyle, were coloured.

In no instance could I detect the colour of the dye used, either in the thoracic duct, lacteals, or lymphatics.

It is reasonable to suppose that had the dyes been absorbed by the lymphatics and lacteals, and not by the veins, they would have been as readily seen in the thoracic duct as they had previously been in the frog's heart, or even more plainly.

Leaving for the present what I have to say of the blood, I pass to the microscopic examination of the other fluids and tissues.

I have already mentioned the dyes employed, and it only remains to state, that they were of all degrees of concentration, from their undiluted condition to the proportion of one part of dye to twenty of water. Generally they were injected into the peritoneal cavity, or beneath the skin, and once into the cavity of the pleura. Sometimes their temperature was raised to that of the blood of the animal; at other times they were injected cold. The dye usually appeared in the urine a few minutes after injection, and half an ounce of the dye—a quantity sufficient to make a gallon of ordinary dye—*passed entirely out of the body in*

twenty-four hours As might have been expected, absorption and excretion were more rapid in the fowl than in the dog.

It would be tedious to read the notes of all the experiments, but any one wishing to do so, may at any time see them.

The following fluids and tissues of animals, frogs, birds, and dogs, were microscopically examined when it was believed that most dye was present in the body :

Chyme, chyle.

Fluid of mesenteric glands and lymphatics.

Voluntary and involuntary muscular fibre.

Muscular fibres and lining membrane of heart.

Brain, spinal-cord, and nerves.

Cartilage, bone.

All epithelial structures.

Liver, lung, spleen, and kidney.

In none could I detect the slightest evidence of colour.

We are then satisfied that although the dye permeates every atom of the body, excluding only the densest tissues, its presence cannot be discovered microscopically in any. Is it then absorbed by the blood discs, or does it become an undistinguishable part of the serum ?

MICROSCOPICAL EXAMINATION OF THE BLOOD.

Many difficulties lie in our way, some of which may be considered insurmountable.

1st. The evidence of colour is very untrustworthy, depending as it does upon the power of appreciation of the observer, and it becomes much more so when the subject of observation is a microscopic particle, something requiring a quarter inch objective to discern and describe. It varies also with the colour of the light transmitted, as we use a white cloud, blue sky, or light from a lamp.

2nd. Too much stress must not be laid on the very minute quantity of colour necessarily present in any particle so small as a blood corpuscle, for the dye is readily visible when really absorbed, in particles very much smaller.

3rd. By far the greatest difficulty lies in the diffracting power of the red corpuscles, whereby the rays of light are made to interfere, and this is especially the case when the blood having been first dispersed with the point of a scalpel over the slide, is allowed to dry. It constantly happens that

they are seen separated at equal distances from each other, and being perfectly circular, show the most beautiful prismatic colours when *attentively* examined with a good quarter inch objective. The colours change with the distance of the objective from the discs, exactly as they do when a piece of perforated zinc held in a beam of diverging light is looked at through a lens, and the distance varies between the zinc and the lens. The appearances presented I have here sketched, and I wish to add, that I am indebted to my colleague, Professor Wilson, for a great deal of information on this curious subject of interference, and to Mr. Ellery, for having taken a great deal of trouble to examine many specimens of blood.

4th. It follows, that the observer in his desire to succeed, can find almost any colour he is seeking for, and not in the blood-disks alone, but in those microscopic globules at times so abundant in the blood, according as the distance between the disks and the objective is varied; and as the red is the broadest of these interference fringes, it and its complementary colour green, are most readily seen.

I have then to state, that a drop of blood from an animal injected as above, gives no certain evidence of its presence in either the white corpuscle, lymph, or chyle corpuscle; at most they are all very slightly tinged of whatever colour has been injected. Thus, the red spot seen in the centre of the dried disk, when in focus, is more brilliant when magenta has been injected, but more than this I cannot say. I have never seen the nucleus of the white and allied corpuscles tinged, although I have experimented upon sucking animals, in whose blood they are so abundant.

It had been found by Dr. Roberts, that after the formation of the "pullulation," if magenta was applied, "the projections were found to take the dye strongly, and especially the vesicular body within the hood."

In order to determine whether the living corpuscles would be similarly affected, I injected half an ounce of tincture of galls mixed with one and a-half ounce of warm water into the peritoneal cavity of a fowl. Within a very few minutes our "feathered biped" showed symptoms of intoxication, and for the space of an hour was ludicrously drunk, reeling and staggering about, but rapidly recovered.

During all this time he was passing tannin in his urine, as was evidenced by testing with a solution of iron. It soon, however, disappeared both from the blood and urine.

Microscopical examination of the blood showed nothing of the pullulation, nor did the subsequent application of magenta reveal any previous action of the tannin.

A similar experiment was performed upon a dog. It would have been impossible to swear in a court of justice whether the dog became drunk or not; having four legs he managed to balance himself tolerably well, but, as before the experiment he was very savage and snappish, and afterwards was very sociable, familiar, and waggy-tailish, I have little doubt that the alcohol floated to the surface those weak points of his character which his habitual beverage kept usually suppressed. Be this as it may, the blood showed no evidence of the action of tannin.

These experiments, although failing in the purpose for which they were commenced, show, if this were now necessary, the facility with which fluids external to the blood-vessels permeate them and are finally removed from the system; absorption taking place principally through the blood-vessels, and not evidently through the so-called but badly-named absorbents, for in no instance were the contents of the thoracic duct tinged. No doubt whatever exists of the absence of the dye in the thoracic duct, whilst its presence first in the blood, then in the urine, is undoubted. Again, we are led to admire the equal facility that exists for the removal of matters absorbed into the blood by the kidneys, a power that is truly marvellous, preserving in all these experiments the entire health of the animal, and preventing even their own epithelium being coloured.

During the absorption of the dyes from the peritoneal cavity, in two or three instances, there was a simultaneous accumulation of colourless serum in the same cavity, a true endos and exosmosis occurring in the same membrane and same vessels at the same time (?).

The foregoing observations are deduced from upwards of one hundred experiments.

I now proceed to the results obtained by injecting solutions of ferrocyanide of potassium and perchloride of iron into the peritoneal and pleural cavities, stomach, and beneath the skin.

Fodera, in 1824, (see Milne Edward's "*Leçons sur la Physiologie*,") by injecting an infusion of galls into the pleural sac or urinary bladder, and at the same time a solution of sulphate of iron into the peritoneal cavity, found a black precipitate in the thoracic duct and other parts of the body.

In other experiments he substituted for the galls a solution of ferrocyanide of potassium, and succeeded in discovering prussian blue in the thoracic duct and elsewhere.

Again, one of our best workers in science, Mr. Ralph, of Kew, near Melbourne, in a paper read before the Medical Society of Victoria, December 6th, 1865, states, that when prussic acid has been either swallowed or inhaled, minute particles or films of prussian blue, may, with the aid of the microscope, be detected in the blood; and along with these, in very many instances, he has observed bodies resembling starch-grains, which polarize, and upon the addition of iodine turn purple. His theory is, "that the prussic acid is more or less neutralized in the blood by the iron present in it, and in proportion to the iron thus withdrawn, there is so much starchy matter set free."

My own results are as follows:

Experiment 1.—At 11, a.m., March 28th, 1866, I injected a solution of ferrocyanide of potassium into the peritoneal cavity, and a solution of perchloride of iron beneath the skin of the back of a fowl.

Purging and passing of urine soon followed. On testing the urine for iron, none was discovered, but abundance of the ferrocyanide of potassium. The tests used were—for the iron, tincture of galls and ferrocyanide; for the ferrocyanide, perchloride of iron. No amorphous prussian blue was found in the blood, but on evaporation numbers of minute crystals were observed with the microscope, some green, many "darkly, deeply, beautifully blue, as some one somewhere sings about the skies." As there are both yellow and blue ferrocyanide of potassium, it was impossible at this stage of the experiment to speak with certainty as to the presence of prussian blue in the blood.

At half-past 12, p.m., made the bird swallow four ounces of the solution of perchloride—seemed to like it. Urine and blood again examined—result as before.

At 9 o'clock, the following morning, the urine gave no trace of either iron or ferrocyanide of potassium, and the bird was quite well. It was now killed.

The contents of the thoracic duct were to the naked eye colourless—no appearance of prussian blue—no evidence either of iron or ferrocyanide upon using the ordinary tests. A drop drying on the microscope yielded the same undetermined crystals as the blood.

Upon touching any part of the peritoneal cavity with a

solution of iron, not the slightest reaction took place; so also when applying a solution of the ferrocyanide to the part beneath the skin, where the iron had been injected, no reaction occurred. In none of the tissues were either found in a free state. The alimentary canal, which had contained iron a few hours before in such abundance, now gave signs of none.

From this experiment we infer, that in twenty-three hours the iron had either entered into very stable combinations in the body, or had passed out of the body in the urine in equal stable combination; or uniting with the ferrocyanide, was actually present in the tissues and fluids in the form of the minute* blue crystals before spoken of as seen in the blood and chyle.

It is not to be supposed that these salts are absent from a tissue because they do not answer to ordinary chemical tests. The spectroscope must be used. Here is an illustration of its use: Mr. Bowman, the distinguished oculist and physiologist, had a patient with double soft cataract; she took twenty grains of carbonate of lithium, seven hours after which one eye was operated on, and the lens removed. The minutest portion showed abundance of chloride of lithium in the spectrum. Seven days having elapsed, the other lens was removed, but not a trace of the salt could be detected. This was a part of a series of experiments recently made by my former teacher, Dr. Bence Jones, to whom I am much indebted for instruction in what may be termed clinical chemistry.

Experiment 2.—One and a-half ounce of solution of perchloride of iron were injected into the peritoneal cavity, and one and a-half ounce of solution of ferrocyanide of potassium beneath the skin of the back of a dog.

At intervals during the succeeding twenty-four hours, I frequently examined the blood for free iron and ferrocyanide, but found none; but beneath the microscope crystals on evaporation, as in the blood of the cock.

Twenty-six hours after the injections a large quantity of urine was passed, to some of which, when a drop or two of perchloride of iron was added, a large quantity of prussian blue was formed. No trace of free iron. A drop of the urine evaporated on a slide showed beneath the microscope an

* When I use the word "minute," I mean something sensibly much smaller than a human red blood-corpuscle.

enormous quantity of bright blue particles. Another portion of the urine was taken, and evaporated in a white porcelain dish; towards the end of the process a dirty blue residuum was formed; to this distilled water was added, and gentle heat of a warm-bath applied. Much organic matter was thus removed, the residuum having all the appearance of prussian blue. Examined with the microscope there were no longer the doubtful crystals, but true amorphous prussian blue.

Seven hours after, more urine just passed was examined, but not a trace of either the ferrocyanide or iron was detected, and upon evaporation no prussian blue was obtained.

Thus, then, in twenty-four hours were the kidneys enabled to remove this large amount of iron and potash from the body; a result agreeing with what we saw in the last experiment with the cock, and with all the previous ones with the aniline dyes.

Experiment 3.—Three days after this, the dog being perfectly well, on again tapping the abdomen, I drew off some serum slightly tinged with blood. In this I sought to detect some of the iron I had three days previously injected; not a trace was discovered. I again threw in one and a-half ounce of solution, perchloride of iron, raised to 100° F., and one and a-half ounce of a saturated solution of ferrocyanide of potassium of the same temperature. The former into the peritoneum, the latter beneath the skin of the back.

During the succeeding twelve hours, the blood yielding crystals as before, the urine was several times examined, giving still more abundant evidence of the presence of the ferrocyanide, but not of the iron, until evaporation, when the residuum being washed, prussian blue, this time in great quantity, was obtained. Besides the peculiar colour of prussian blue, and its amorphous appearance beneath the microscope, the residuum behaved as follows:—

1st. Insoluble in water.

2nd. Colour destroyed by sulphuric acid, and reproduced on the addition of water.

3rd. Both potash and soda bleached it, but the colour reappeared on the addition of either sulphuric or hydrochloric acid.

4th. Oxalic acid changed it to a most beautiful blue, but failed to dissolve it, probably owing to the organic matter with which it was, doubtless, still combined.

Twelve hours after a large quantity of paler urine was passed, in which not a trace of either salt injected was discovered.

Forty-eight hours after the dog had been first injected it was killed.

There were about two ounces of bloody serum in the abdomen.

Not a trace of iron or potash were found in this serum or in any of the tissues.

No prussian blue in the thoracic duct or elsewhere.

Experiment 4.—Injected one and a-half ounce of warm saturated solution of ferrocyanide of potassium beneath the skin of the back of a large dog. Some hours after urine was voided, containing abundance of the salt injected. This upon evaporation yielded yellow crystals. No trace of blue.

Experiment 5.—Saturated some human urine with ferrocyanide of potassium. On evaporation crystals, as in the last experiment. No blue colour.

It thus appears that when iron is introduced into the system, it passes off by the kidneys in combination with some other constituent, from which it is only separated by heat, when, if the ferrocyanide of potassium is present, prussian blue is immediately formed. It does not appear that the ferrocyanide of potassium is in any other state than solution. Neither of the salts were ever detected in the fæces; the same is true of the aniline dyes.

It is known that ferrocyanide of potassium, by exposure, assumes a blue tint, yet is still crystalline. Experiments 4 and 5 were instituted to see if, by the process of evaporation, a similar colour could be produced, so as to cause a doubtful result. They prove clearly the presence in Experiments 1, 2, and 3, of a far larger amount of iron than is contained in any ferrocyanide, sufficient indeed to produce the well-known amorphous prussian blue of commerce.

Whilst these experiments show no such combination takes place within the body, still they reveal the presence of a salt in the blood, chyle, and urine, which upon evaporation at ordinary temperatures appears as minute bright blue crystals.